

## cdDNA Sequence and Predicted ORF of FLJ32028 Gene

(SEQ ID NO. 1) N Q A P R A A L V F A L V I A L V P V G R G N Y E E L E N S G D F

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**HindIII**

**Bpu10I**

**BpuII**, **BpuII'**

**BssHII**

**BbsI**

**BaeI'**

**EagI**

**PstI**

**BtsI**

**BspEI**

**BsaBI**

**BaeI**

**PstI**

**BtsI**

**F1 Primer** →

Signal Peptide

TM Region

TM Region

150

300

450

IP] P [G] P [G]

IP] V E S E R P N K V T I P S T F A A V T I K E T L N A N I N S T N F A P D E N Q L E F I L M V L I P

IP] P [G] P [G]

IP] V E S E R P N K V T I P S T F A A V T I K E T L N A N I N S T N F A P D E N Q L E F I L M V L I P

NdeI

BmrI

IP] L I L V L L L S V V F L A T Y Y K R R T K Q E P S S Q G S Q S A L Q T Y E L G S E N V K V P I

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FIG. 1 can't

## Alignment of Human FLJ32028 Protein with Similar Proteins from Rodents

Sequence alignment diagram showing multiple protein sequences from various species. The sequences are aligned horizontally, with gaps indicated by dashes. A vertical scale on the left shows positions 10, 20, 30, 40, 50, 60, 70, and 80. A horizontal scale at the bottom shows positions 182, 180, 348, 223, 247, 278, 360, 370, 350, 330, 320, 310, 290, 220, 150, 120, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, and 0. Specific amino acid residues are highlighted in boxes, and some are underlined. Reference numbers and sequence IDs are listed along the right side.

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FLJ32028 protein with N-terminal HA Tag

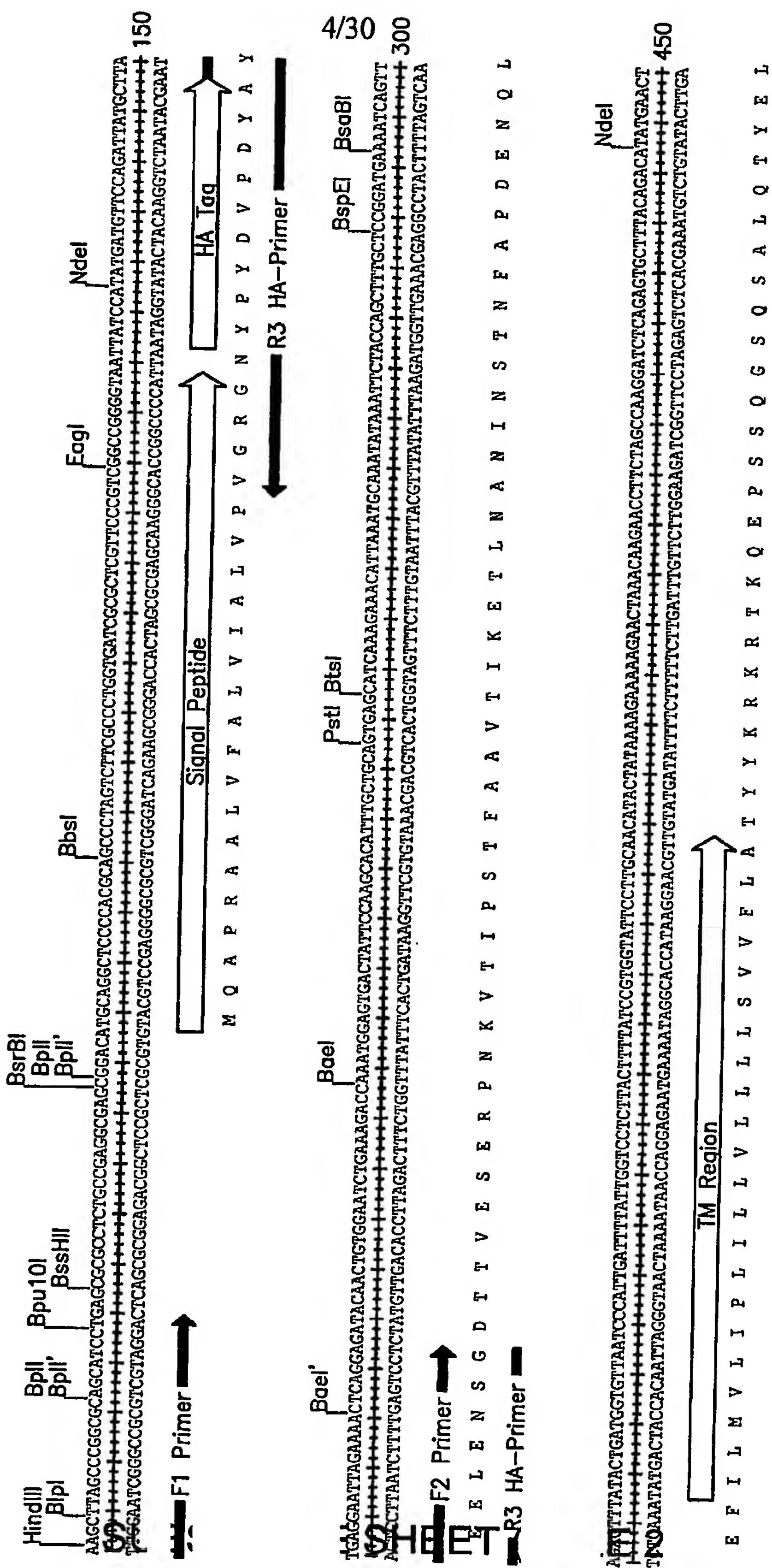


FIG. 3

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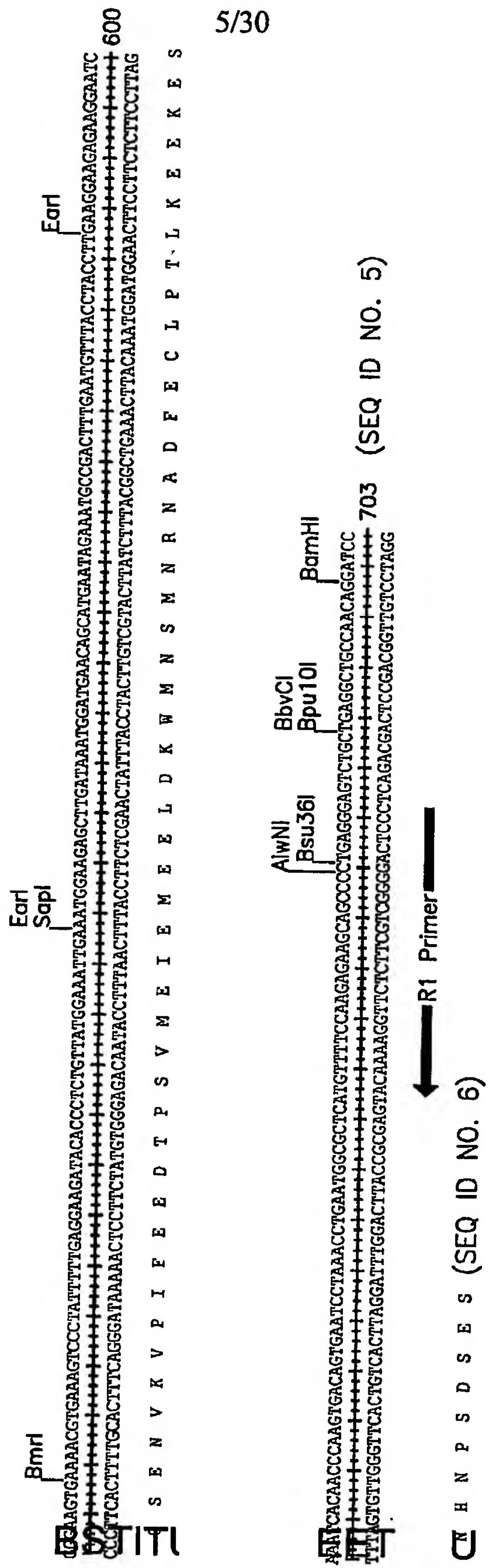


FIG. 3 con't

## FLJ32028 Protein with C-terminal HA Tag

FIG. 4

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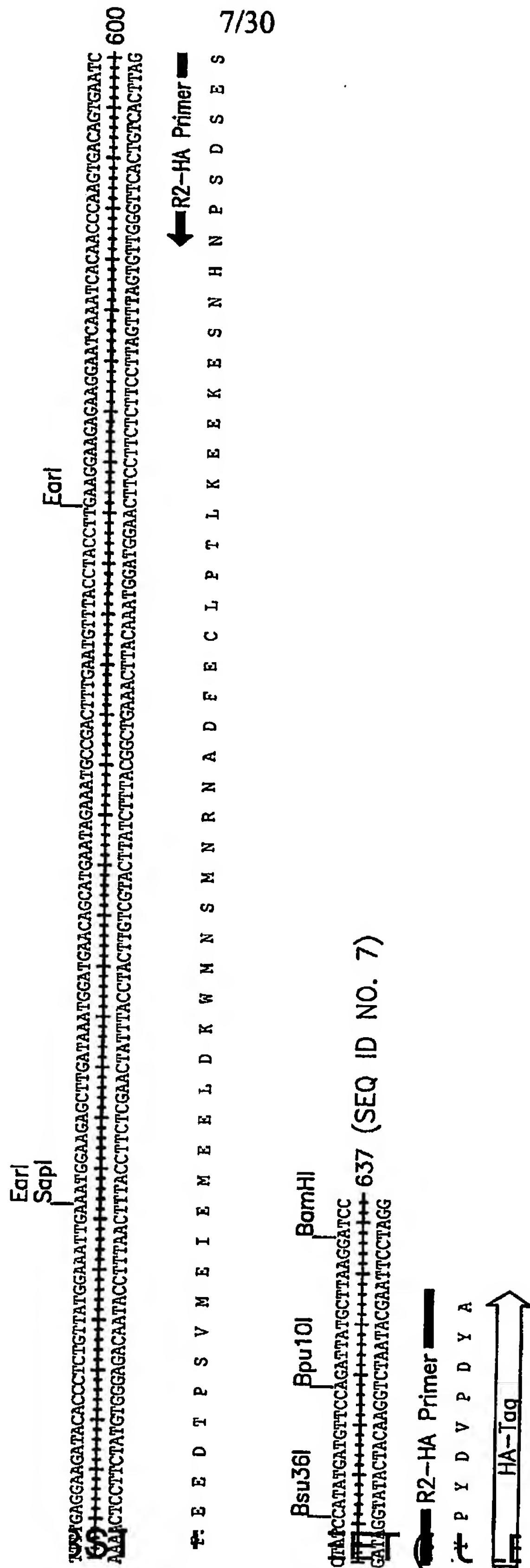
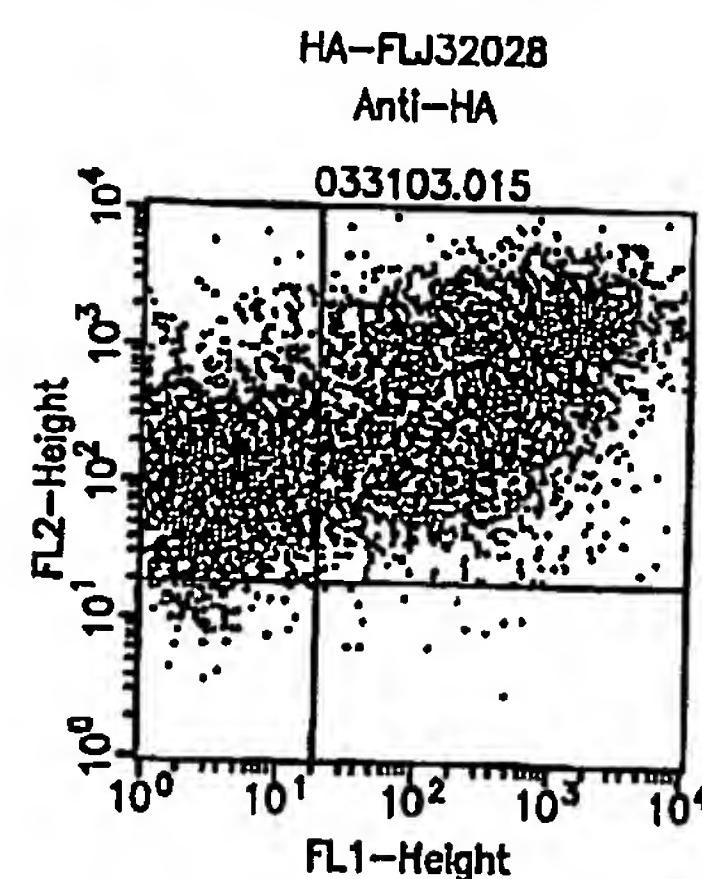
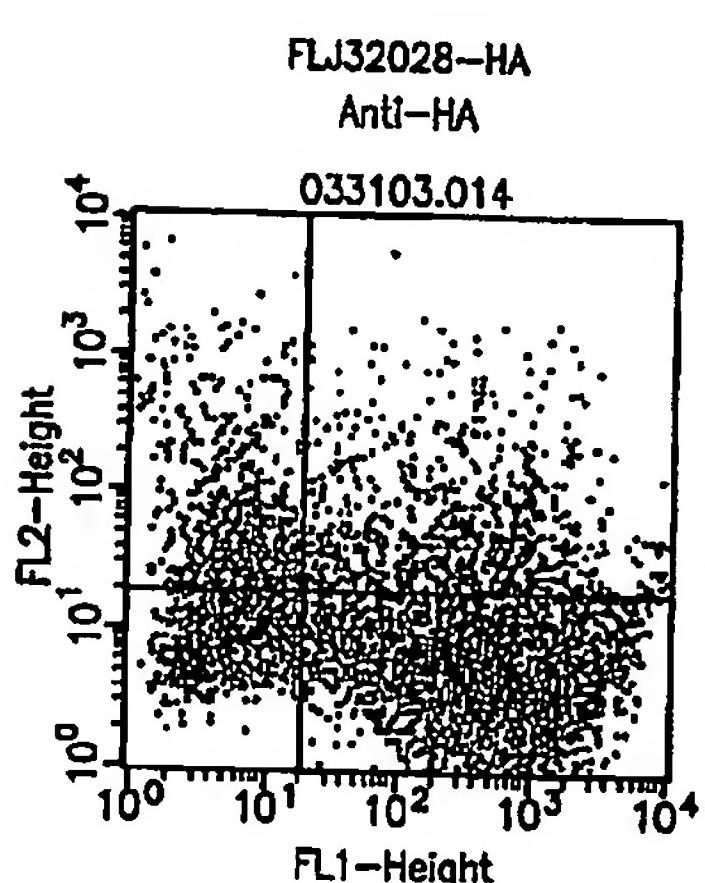
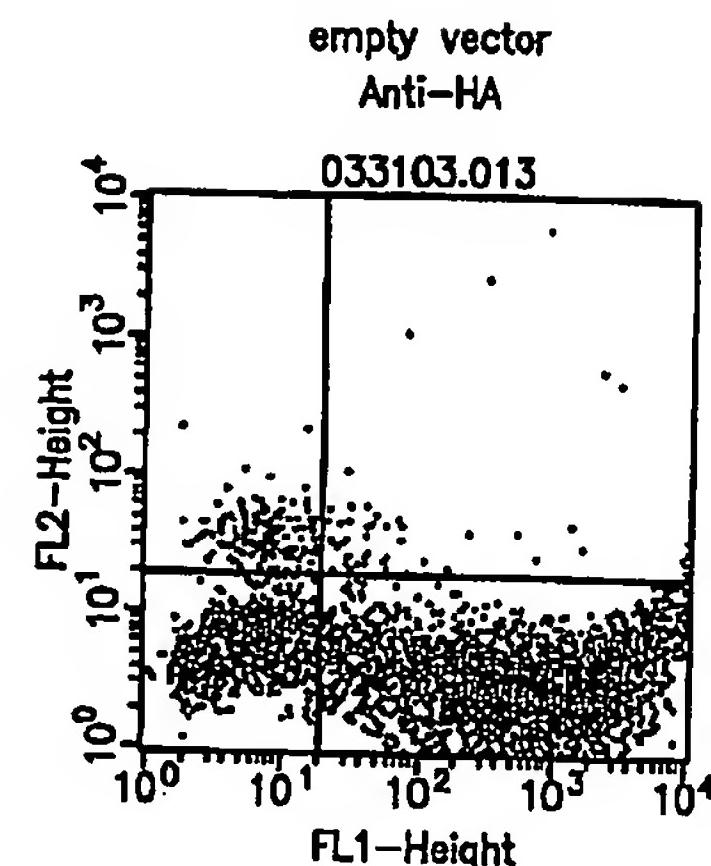
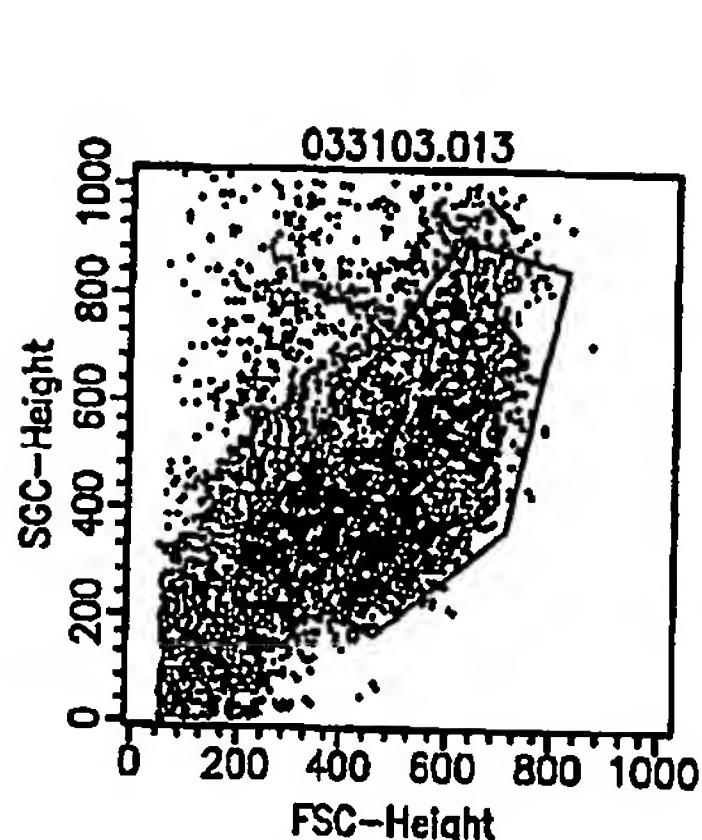


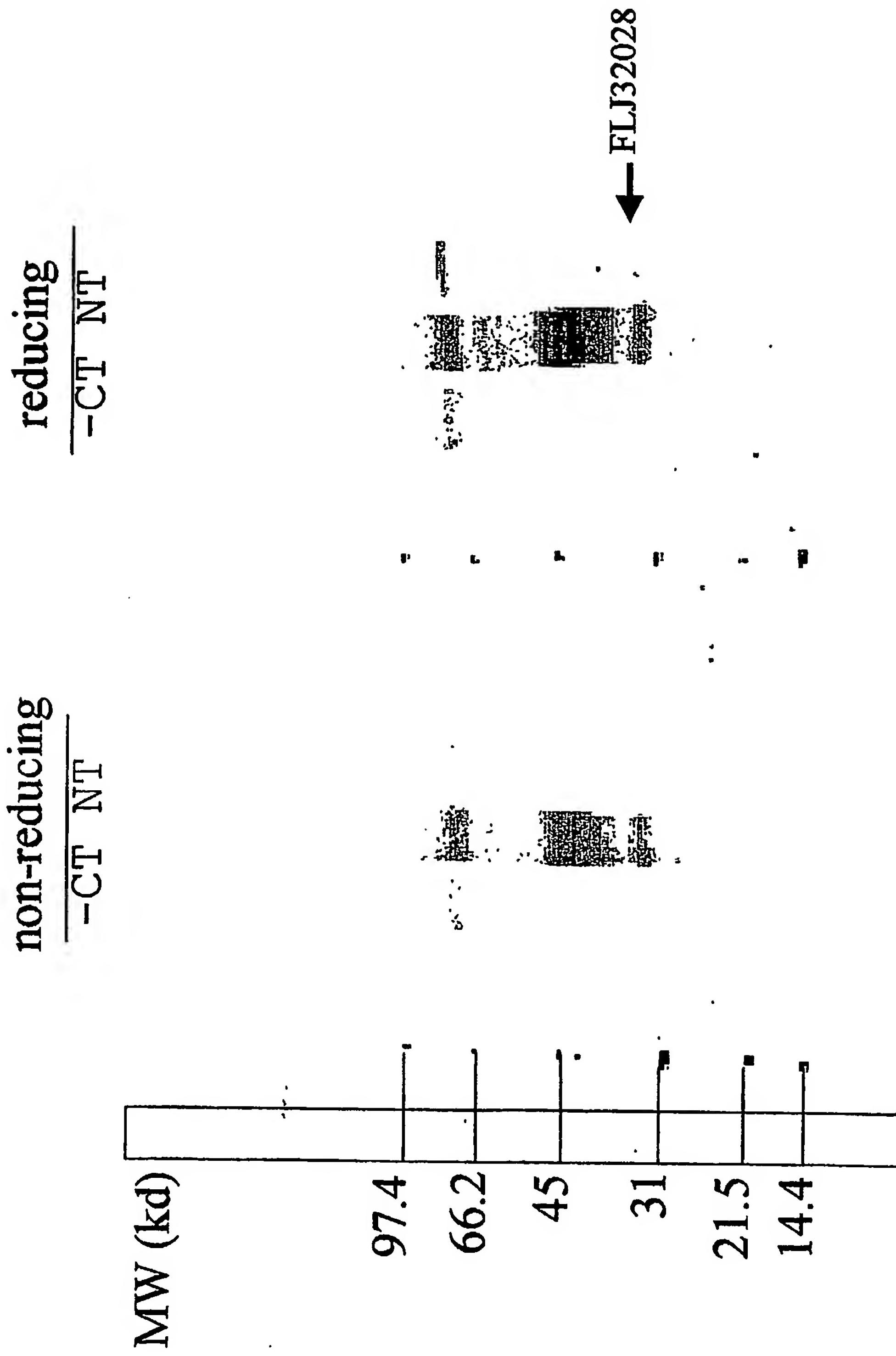
FIG. 4 con't

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## FACs Analysis of Transfected 293-EBNA Cells

**FIG. 5**

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**Anti-HA Western Blot of Transfected 293-EBNA Cells***FIG. 6*

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FLJ32028 polynucleotide sequence (SEQ. ID No. 2):

AGCCCGGCGCAGCATCCTGAGCGCGCCTCTGCCGAGGCAGCGGACATG  
CAGGCTCCCCGCGCAGCCCTAGTCTCGCCCTGGTGATCGCGCTCGTCC  
CGTCGGCCGGGTAAATTATGAGGAATTAGAAAACTCAGGAGATAACTGT  
GGAATCTGAAAGACCAAATAAAGTGACTATTCCAAGCACATTGCTGCAGTG  
ACCATCAAAGAACATTAAATGCAAATATAAATTCTACCAACTTGCTCCGGA  
TGAAAATCAGTTAGAGTTATACTGATGGTGTAAATCCCATTGATTTATTGG  
TCCTCTTACTTTATCCGTGGTATTCTTGCAACATACTATAAAAGAAAAAGA  
ACTAACAAAGAACCTCTAGCCAAGGATCTCAGAGTGCTTACAGACATATGA  
ACTGGGAAGTGAAACGTGAAAGTCCCTATTTGAGGAAGATAACCCCTCT  
GTTATGGAAATTGAAATGGAAGAGCTTGATAAAATGGATGAACAGCATGAATA  
GAAATGCCGACTTGAATGTTACCTACCTTGAAGGAAGAGAAGGAATCAAA  
TCACAACCCAAGTGACAGTGAATCCTAACCTGAATGGCGCTCATGTTTCC  
AAGAGAAGCAGCCCTGAGGGAGTCTGCTGAGGCTGCCAACAA

**FIG. 7**

FLJ32028 polypeptide sequence (SEQ. ID No. 1):

MQAPRAALVFALVIALVPVGRGNYEELENSGDTTVESERPNKVTIPSTFAAVTIK  
TLNANINSTNFAPDENQLEFILMVLIPLILLVLLLLSWFLATYYKRKRTKQEPSSQ  
GSQSALQTYELGSENVKVPIFEEDTPSVMEIEMEELDKWMNSMNRNADFECLP  
TLKEEKESNHNPSDSES

**FIG. 8**

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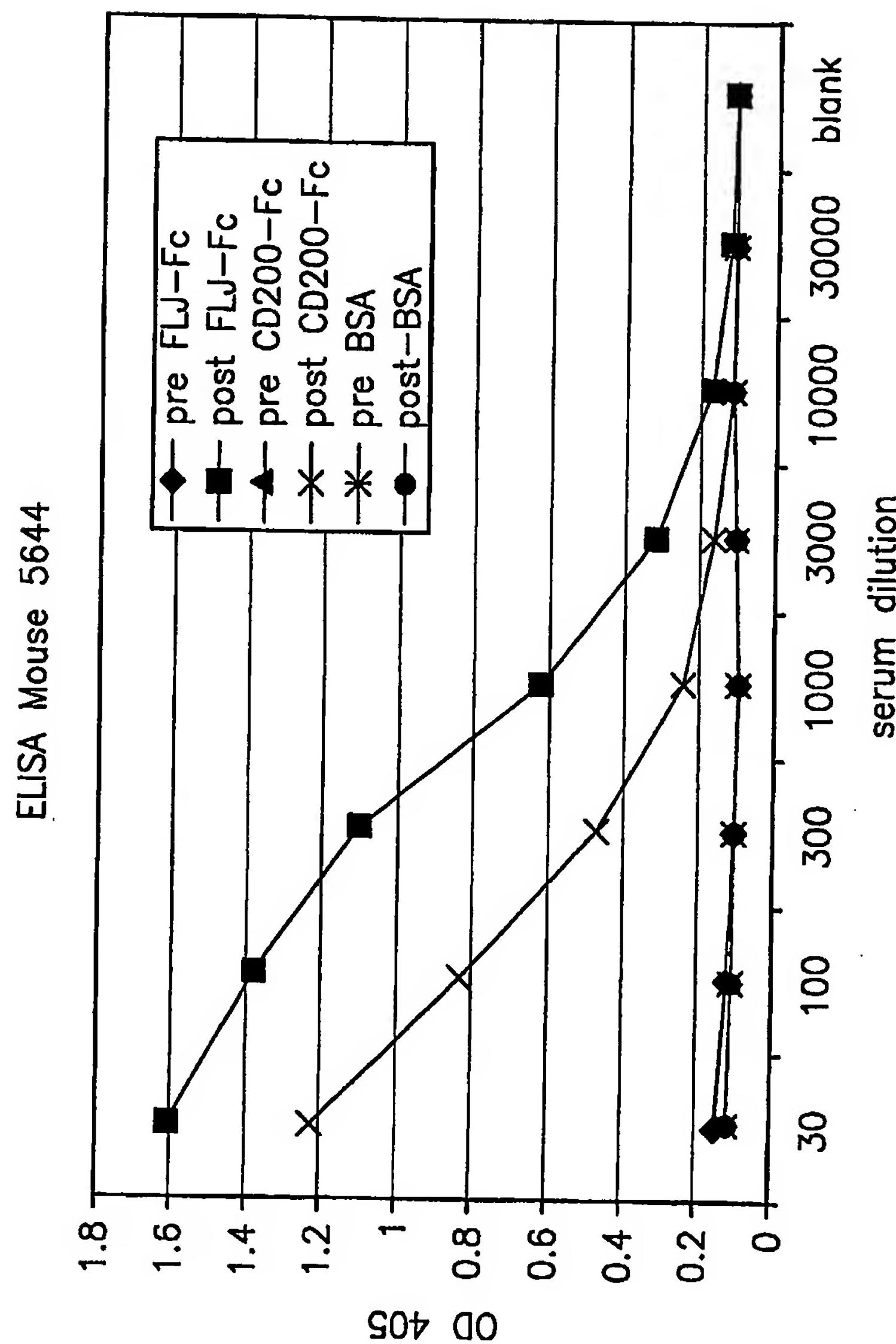


FIG. 9A

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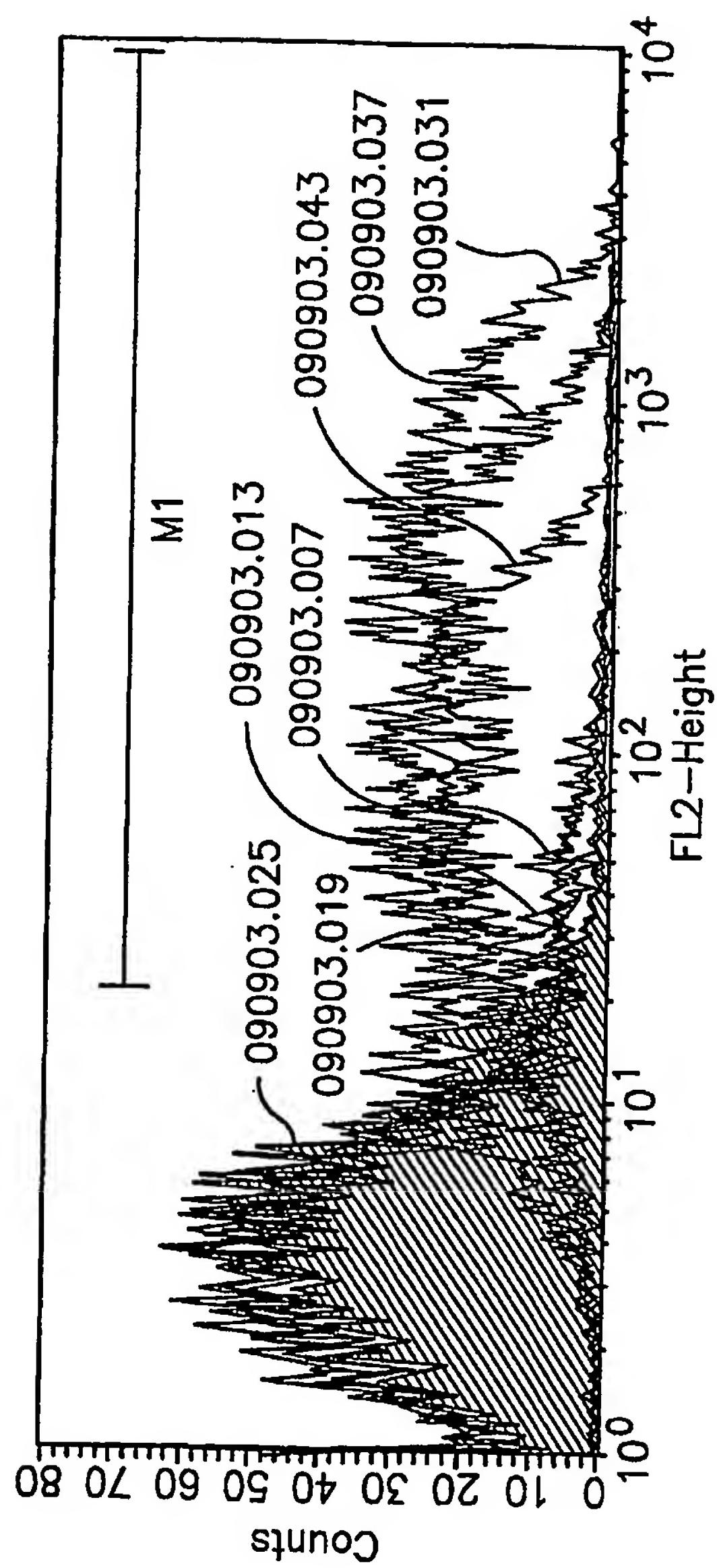
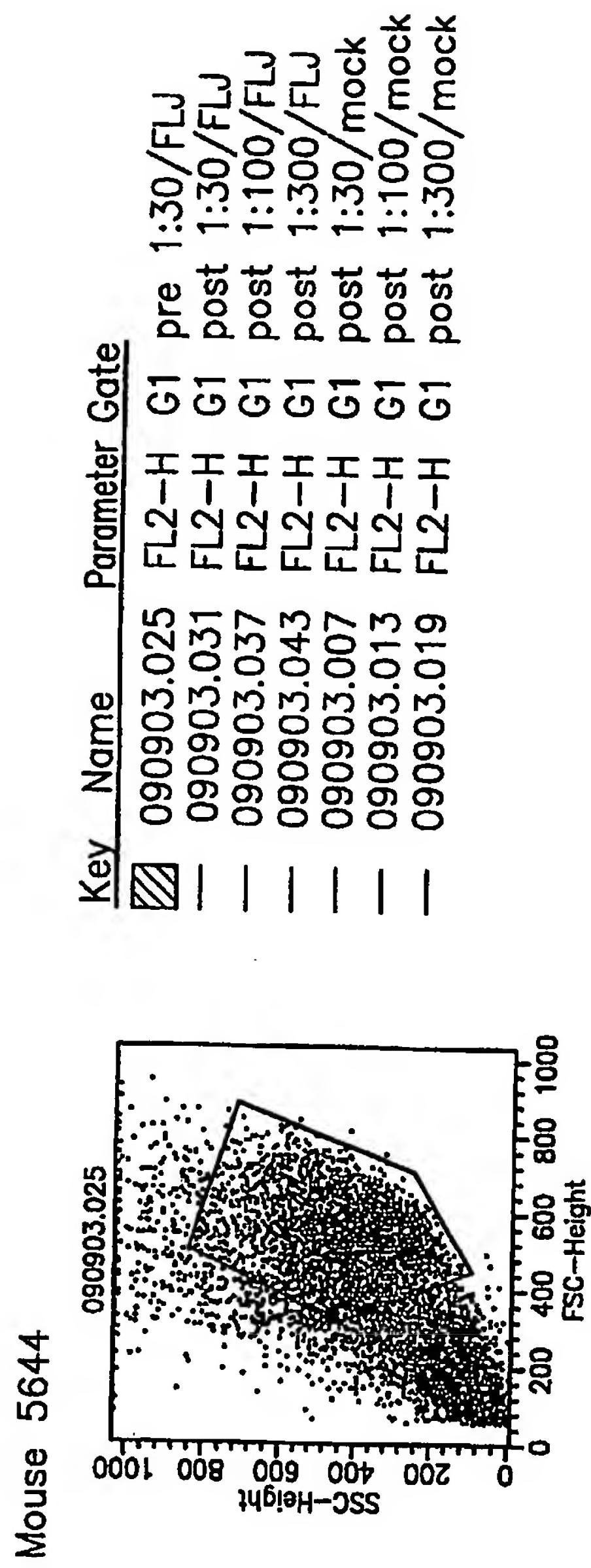


FIG. 9B

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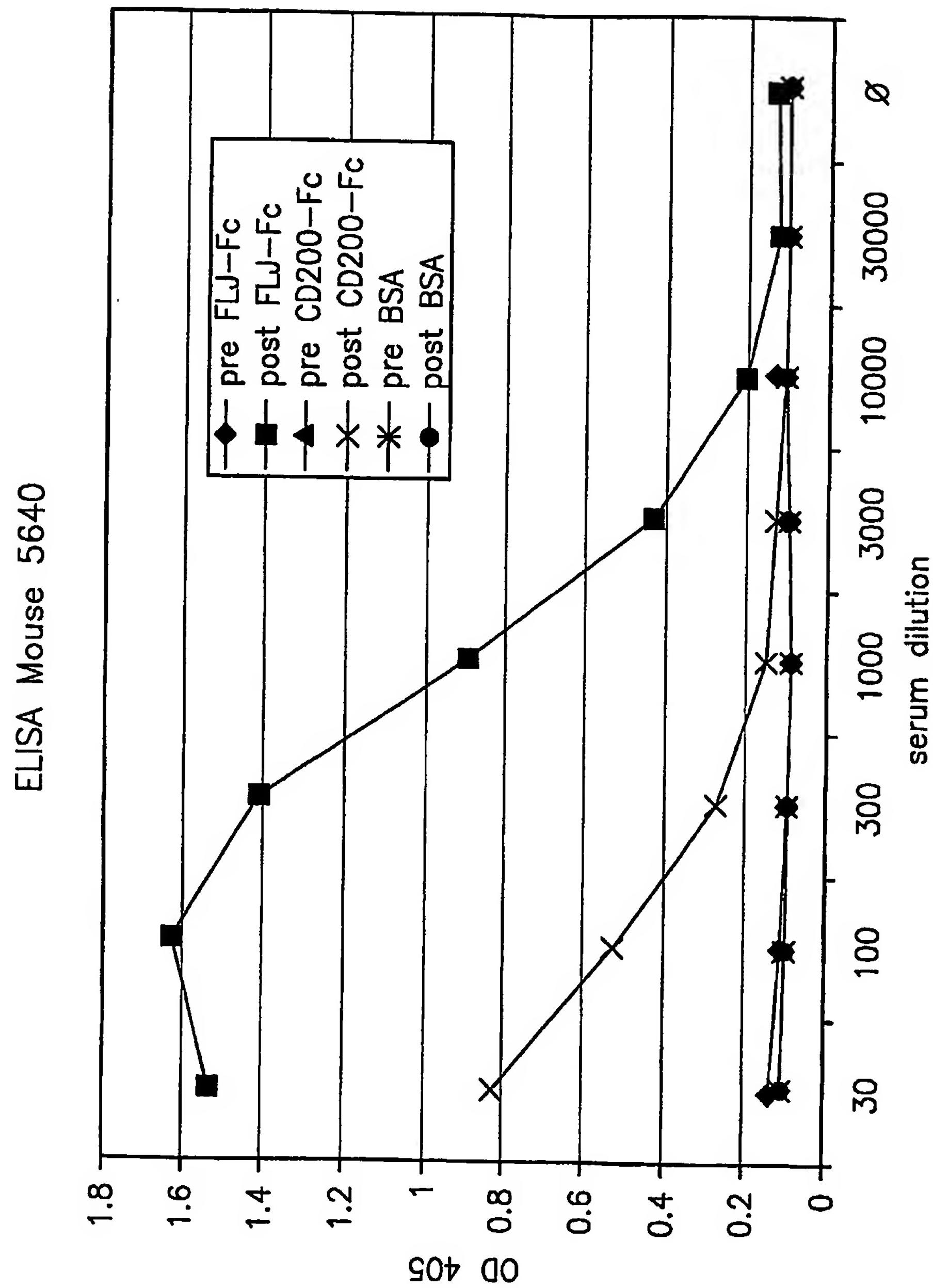
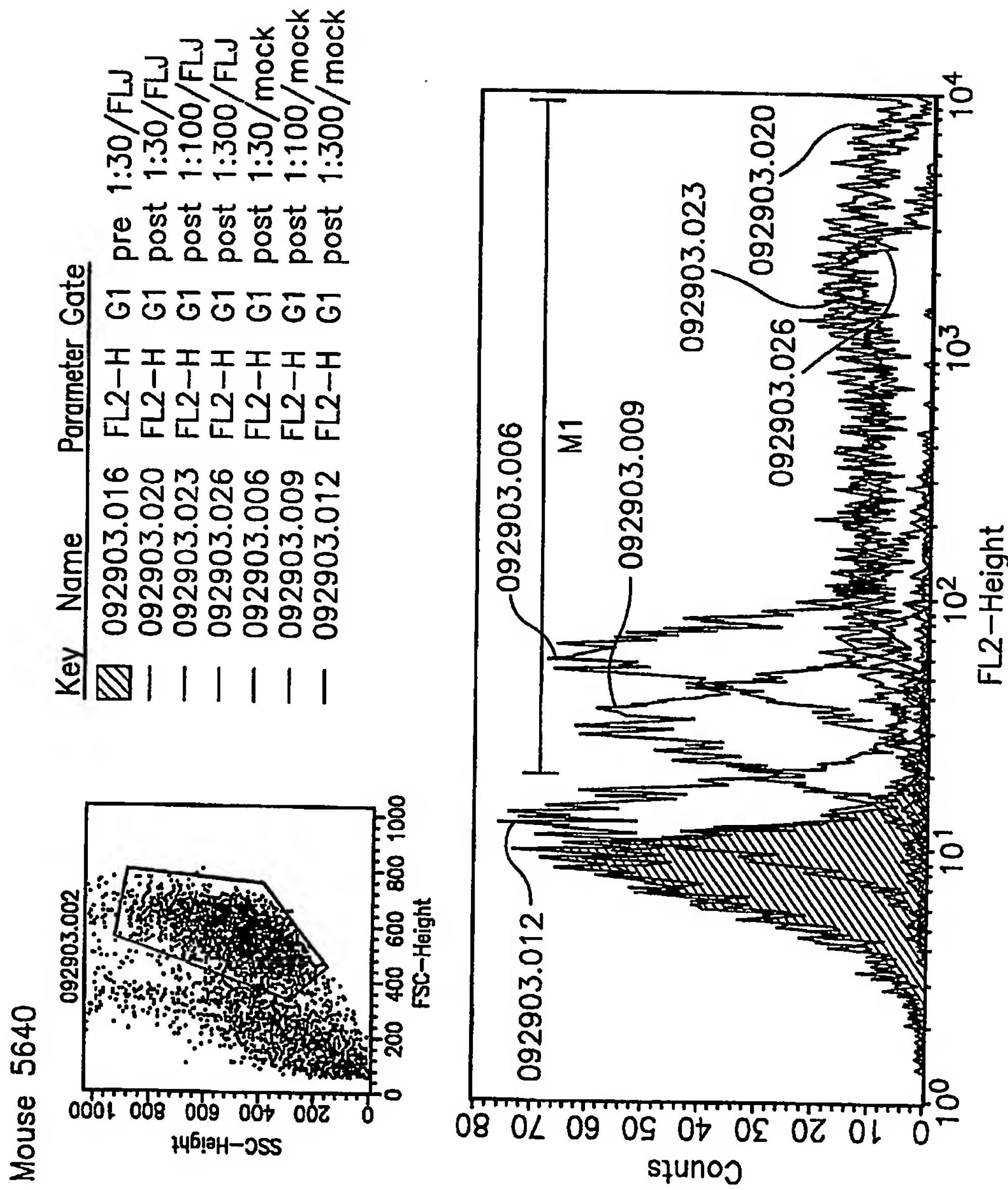


FIG. 10A

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**FIG. 10B**

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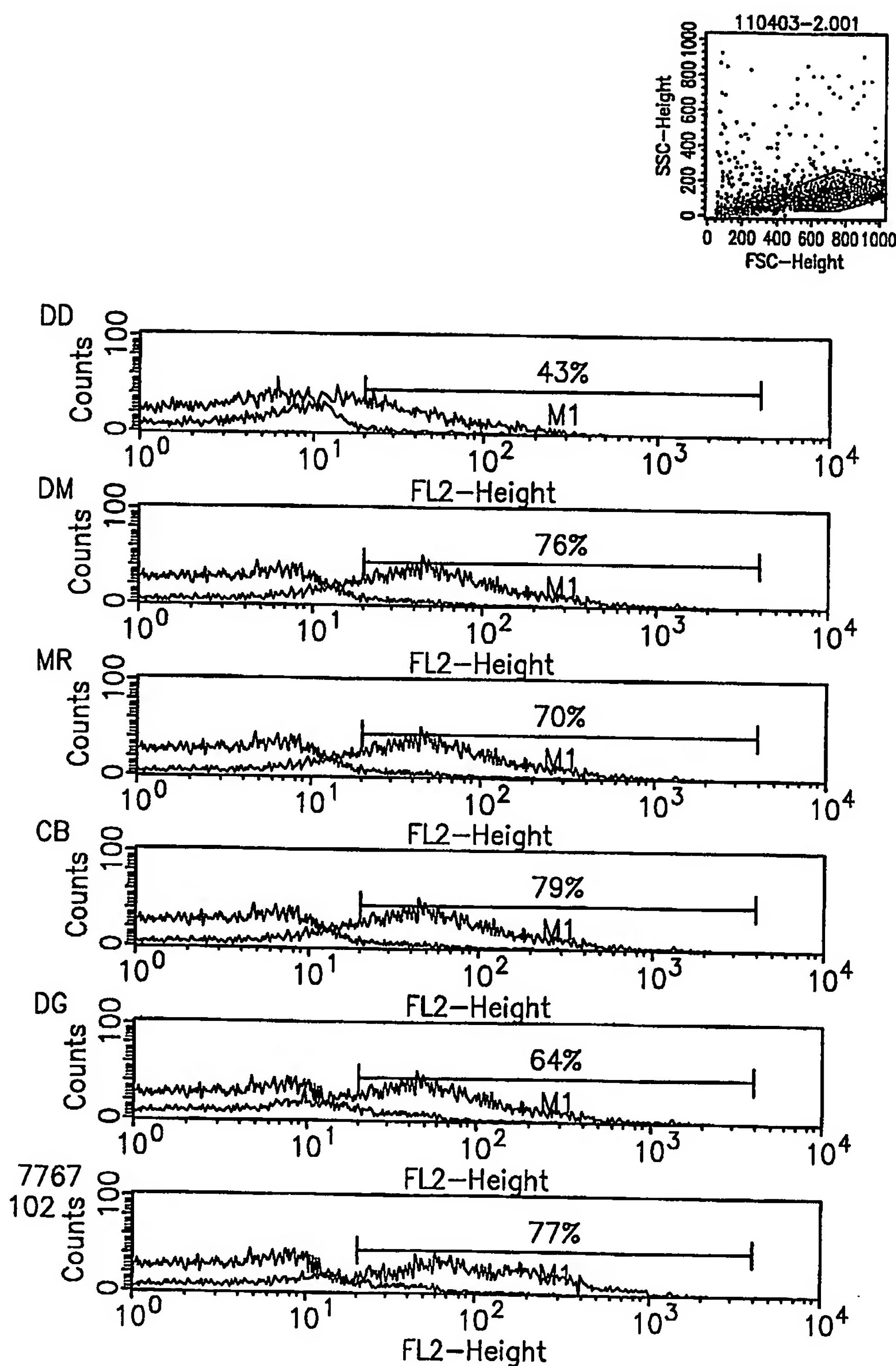


FIG. 11

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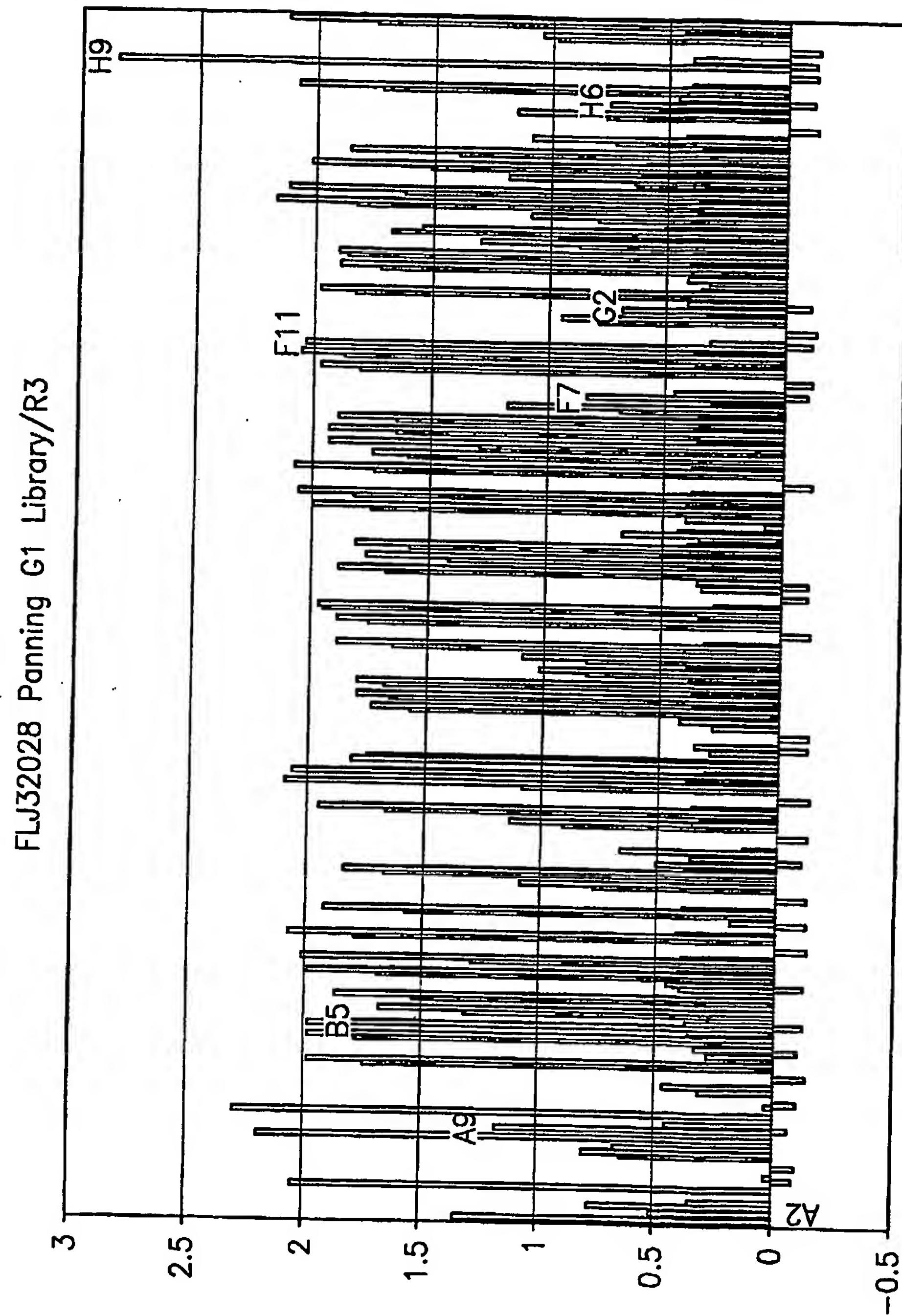
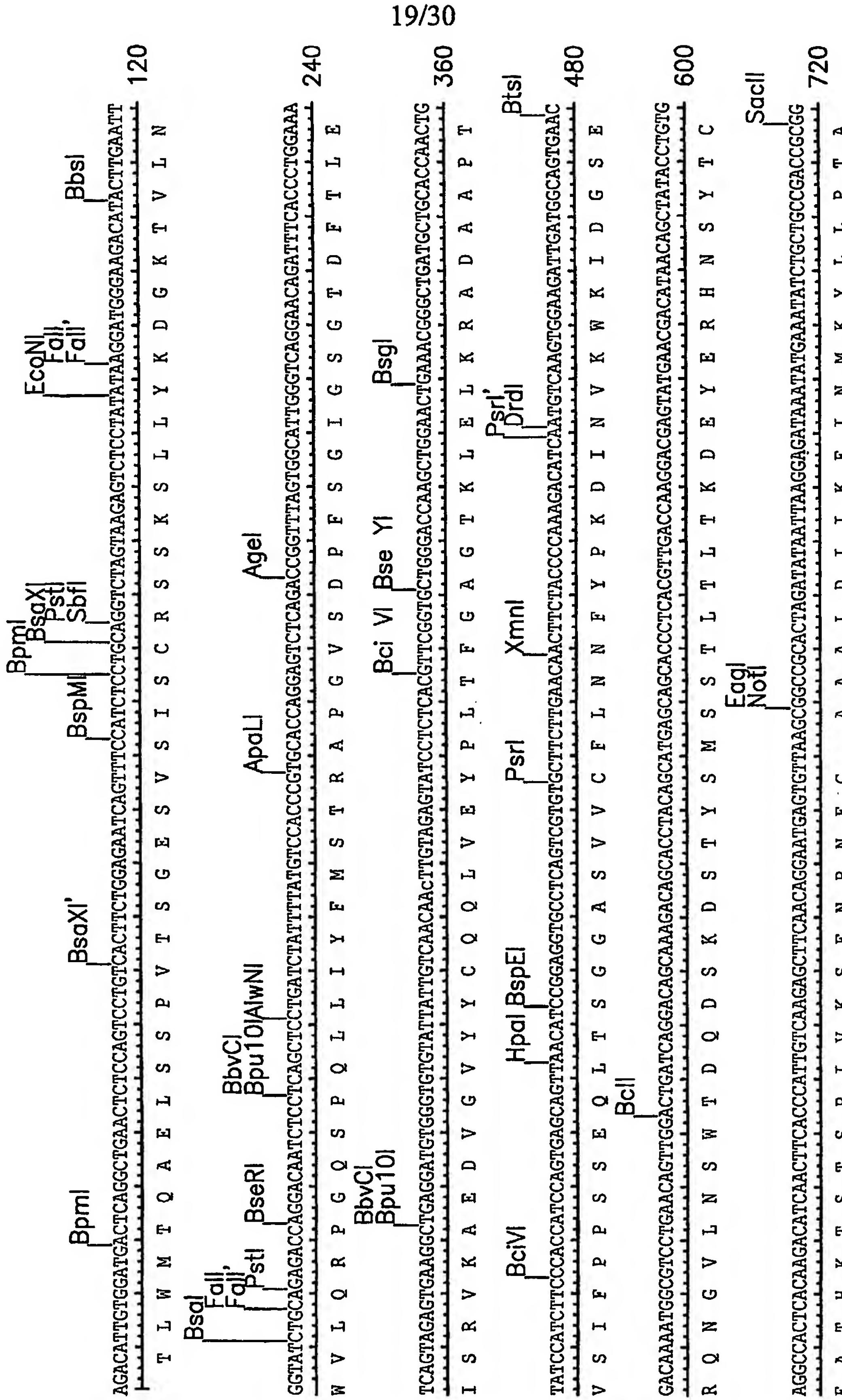


FIG. 12

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BpmI | Acc65I | KpnI | XbaI  
 PflMI | EarI | BpmI |  
 BsdI | Psrl | BsmBI | Bmrl |  
 TCACCTTCACTGCCATGCTGGTCTGGACTCCAGAGAAGCTGCGCATATTAGTAGTGTGAAGACAGTCGGAT  
 960  
 F T F S D Y A M S W V R Q T P E K R L E W V A S I S S G G T T Y Y L D S V K G R  
 BsrMII | Psrl |  
 TCACCATCTCCAGAGATAATGCCAGGAACATCCTGTACCGCATTGTGAGCAGTCAACTGGCACACGGCACACGGCA  
 1080  
 F T I S R D N A R N I L Y L Q M S S L R S E D T A M Y Y C V R S E T N Y W G Q G  
 BbvCI | Bpu10I | BseRI | AvrII | FaiI | FaiI | EcoNI |  
 CCACACTCACAGTCTCCTCAGCCAAACGACACCCCCATCTGCTCTGGATCTGCTGGACTACTCCACTGGCCAAACT  
 1180  
 T T L T V S S A K T T P P S V Y P L A P G S A A Q T N S M I T L G C L V K D Y F  
 BbvCI | Bpu10I | BstEII |  
 Bsu36I | ApaLI |  
 FaiI | Tth111I | CCGAACCGGTGACGGTGTGGAACTCAGCGCTCTGACCAGCGGTGACCCCTCAGTCTACTCC  
 1200  
 P E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S  
 BstXI | BseYI | Alel |  
 GCAGCTGGCACCCAGACCTACATCTGCAACGTTGAGCCCCAACGAAACCAAGCTGACAAAACTAGT  
 1320  
 S S L G T Q T Y I C N V N H K P S N T K V D K K T S → 1421  
 (SEQ ID No: 9)  
 (SEQ ID No: 10)

## **SUBSTITUTE SHEET (RULE 26)**



## **SUBSTITUTE SHEET (RULE 26)**

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VL amino acid sequences of FLJ32028-specific IgG1 kappa clones from 5644 library

Clone	FR1	CDR1	FR2	CDR2
11F	DIQMTQTSSLSASLIGDRVTISCRIS	QDISN-----Y	LNWYQQKPDGTVKVLIY	YTTS
11G	..V...AEL.SPVTS.ES.S...S.	KSLLYK-DGKT.	...L.R.GQSPQL...	FM.
6H	..V...SP...AV.V.EK..M..KS.	.SLLYSSNQKN.	.A.....GQSP.L...	WA.
9H	..V.S.SP...AV.V.EK..M..KS.	.SLLYSSNQKN.	.A.....GQSP.L...	WA.
9A	..VV...PL..PV...QAS...S.	.S.VHS-NGNT.	.E..L...GQSP.L...	KV.
2G	..VV...PL..PV...QAS...S.	.S.VHS-NGNT.	.E..L...GQSP.L...	KV.
7F	.VL...PL..PV...QAS...S.	.S.VHS-NGNT.	.E..L...GQSP.L...	KV.

FR3	CDR3	FR4	ELISA ODs:
RHHSGVPSRFSGSSGGTDSITINNIEQEDIATYFC	QQGNNTIIPERTFGSG	TKLEIKR	2.14/0.12/0.46 (SEQ ID NO: 13)
TRAP..SD...I....FT.E.SRVKA..VGV.Y.	..IVEY.L..A.	....L..	1.98/0.14/0.41 (SEQ ID NO: 14)
TRE...D...T....FT...SSVKA..L.V.Y.	..YYSY.L..A.	....L..	0.86/0.12/0.61 (SEQ ID NO: 15)
ARG...D...T....FT...SSVKA..L.V.Y.	..YYSY.L.I.A.	....L..	2.95/0.11/0.56 (SEQ ID NO: 16)
NRF...D...D....FT.K.SRV.A..LGV.Y.	F..SHV.L..A.	....L..	1.30/0.17/0.61 (SEQ ID NO: 17)
NRF...D...D....FT.K.SRV.A..LGV.Y.	F..SHV.L..A.	....L..	0.80/0.12/0.57 (SEQ ID NO: 17)
NRF...D...D....FT.K.SRV.A..LGV.Y.	F..SHV.L..A.	....L..	0.95/0.13/0.62 (SEQ ID NO: 17)

FR: framework region; CDR: complementarity determining region. Dots indicate gaps in the alignment sequence. Dashes indicate identities with the clone 11F sequence. ELISA ODs are given for binding to FLJ32028-Fc fusion protein(FLJ), to negative control Fc fusion protein(Fc), and to Anti-F(ab')<sub>2</sub> (Fab).

FIG. 15

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VH amino acid sequences of FLJ32028-specific IgG1 kappa clones from 5644 library

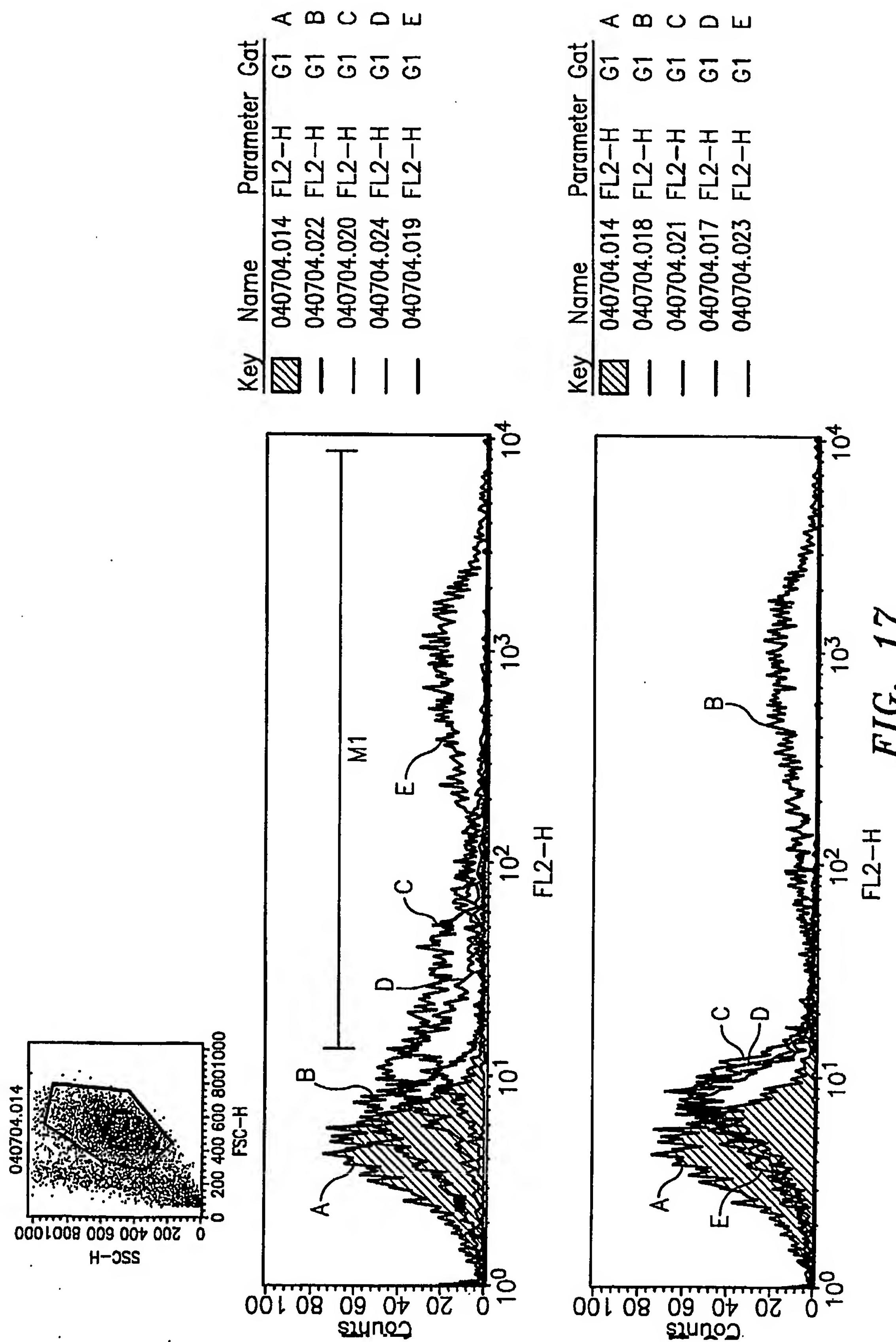
Clone	FR1	CDR1	FR2	CDR2
11F	LEVQLQQSGAELVVRPGASVTISCKAS	GYTFIDYEMH	WKQTPVHGLEWIG	GIDPEIGGTIVYNQKEKG
11G	.....	D.....	.....	.....
5B	.....	.....	.....	.....
6H	...K....K....K....T...	ENIK.TYIN	...R.EQ...	R...ANNN.N.DP..Q..
9H	...F....K....K....TG..	ENIK.TY.N	..I.R.EQ...	M...AN.N.Q.DP..Q..
9A	...K.VE..GG..K..G.LK..A..	F..S..A.S	..R..EKR..	S..S-SG..T.Y.LDSV..
2G	..AK.VE..GG..K..G.LK..A..	F..S..A.S	..R..EKR..	S..S-SG..T.Y.LDSV..
7F	...K.VE..GG..K..G.LK..A..	F..S..A.S	..R..EKR..	S..S-SG..T.Y.LDSV..

FR3	CDR3	FR4	ELISA ODs:
KATITADKSSGTAYMELRSILTSEDAVYYCTS	F---AY	WGQGTLVTVSA	2.14/0.12/0.46 (SEQ ID NO: 18)
...A.S.....A	G---V.	.....	1.98/0.14/0.41 (SEQ ID NO: 19)
...R.....A	G---AD	.....F.	1.84/0.11/0.53 (SEQ ID NO: 20)
...I...TP.N...I.Q.S....TD....V.	GGYFD.	....TL...S	0.86/0.12/0.61 (SEQ ID NO: 21)
...I...T...N...I.Q.S....T....T.	GGYFD.	....TL...S	2.95/0.11/0.56 (SEQ ID NO: 22)
RF.ISR.NARNIL.LQMS..R...T.M...VR	S-ETN.	....TL...S	1.30/0.17/0.61 (SEQ ID NO: 23)
RF.ISR.NARNIL.IQMS..R...T.M...VR	S-ETN.	....TL...S	0.80/0.12/0.57 (SEQ ID NO: 24)
RF.ISR.NARNIL.IQMS..R...T.M...VR	S-ETN.	....TL...S	0.95/0.13/0.62 (SEQ ID NO: 23)

FR: framework region; CDR: complementarity determining region. Dots indicate identities with the clone 11F sequence. Dashes indicate gaps in the alignment. ELISA ODs are given for binding of the phage antibody clones to FLJ32028-Fc fusion protein(FLJ), to negative control Fc fusion protein(Fc), and to Anti-F(ab')<sub>2</sub> (Fab).

FIG. 16

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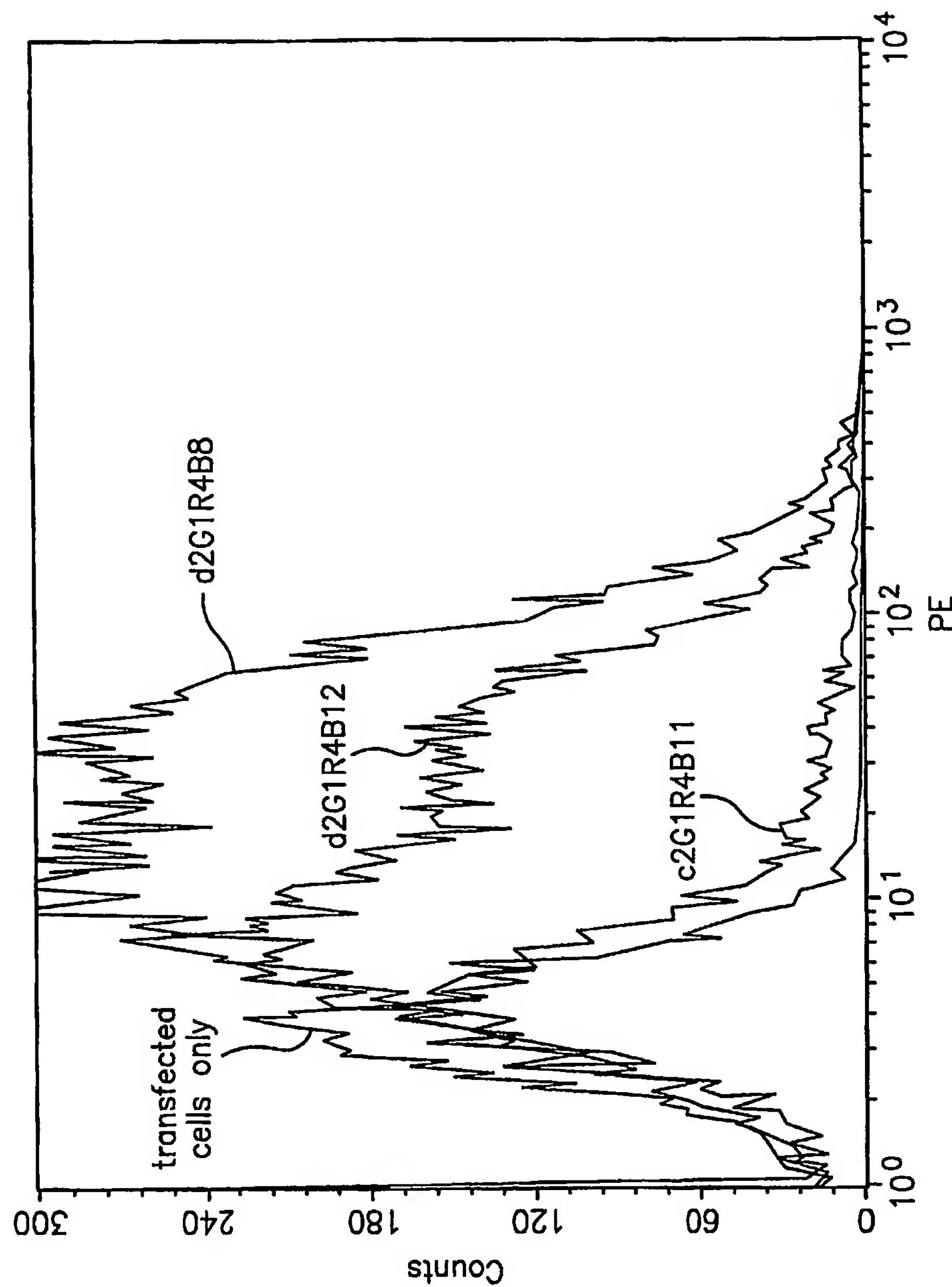


FIG. 18A

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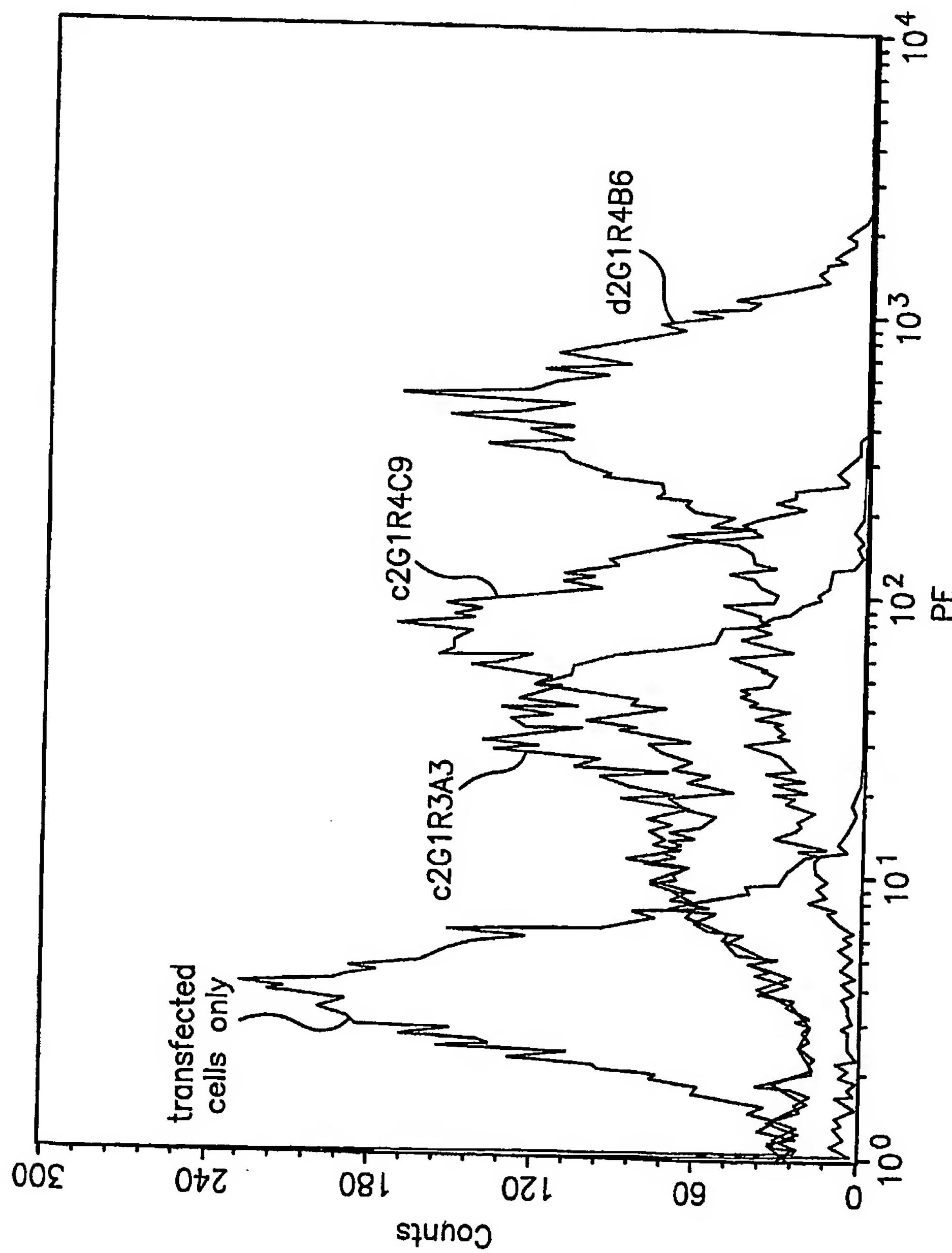


FIG. 18B

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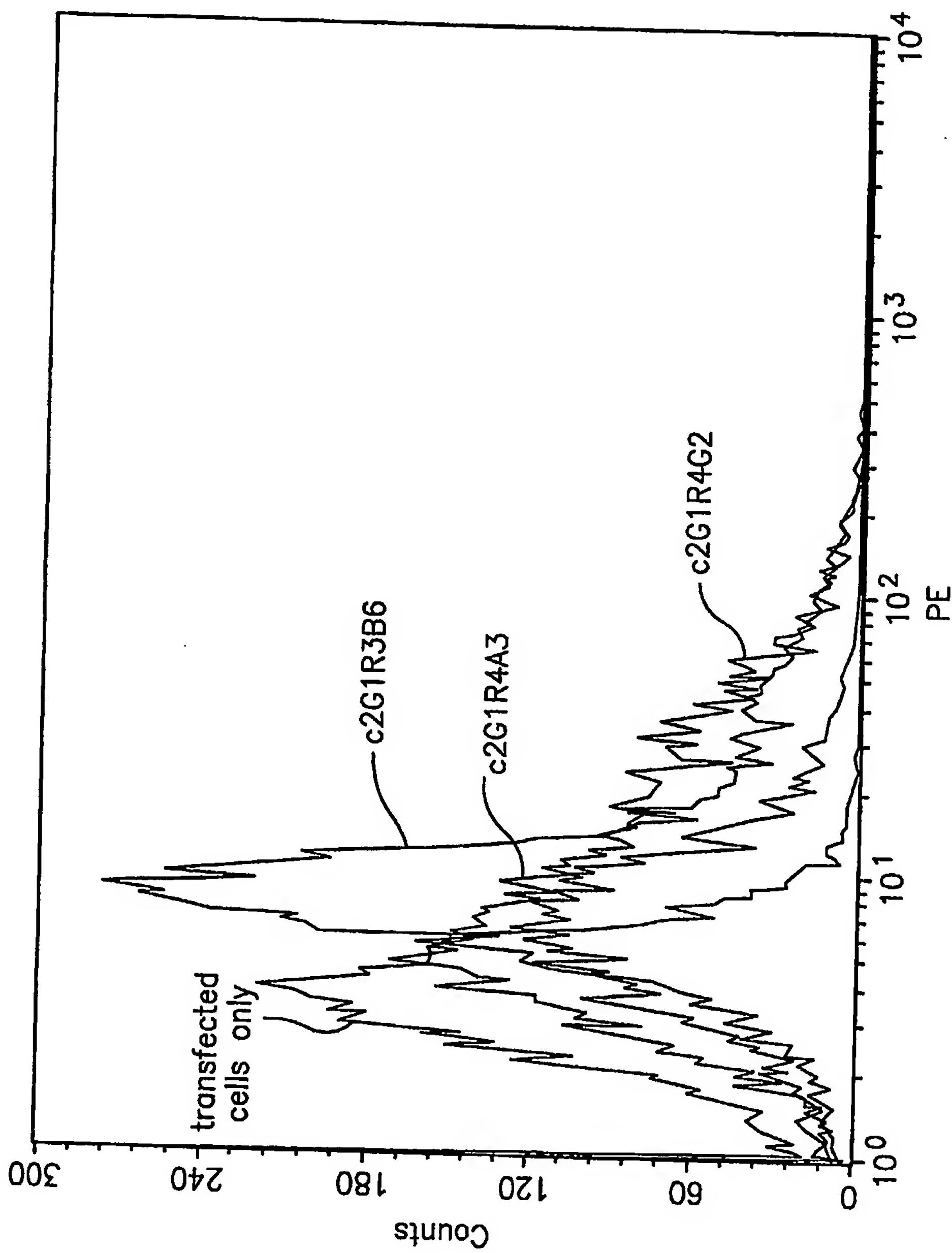


FIG. 18C

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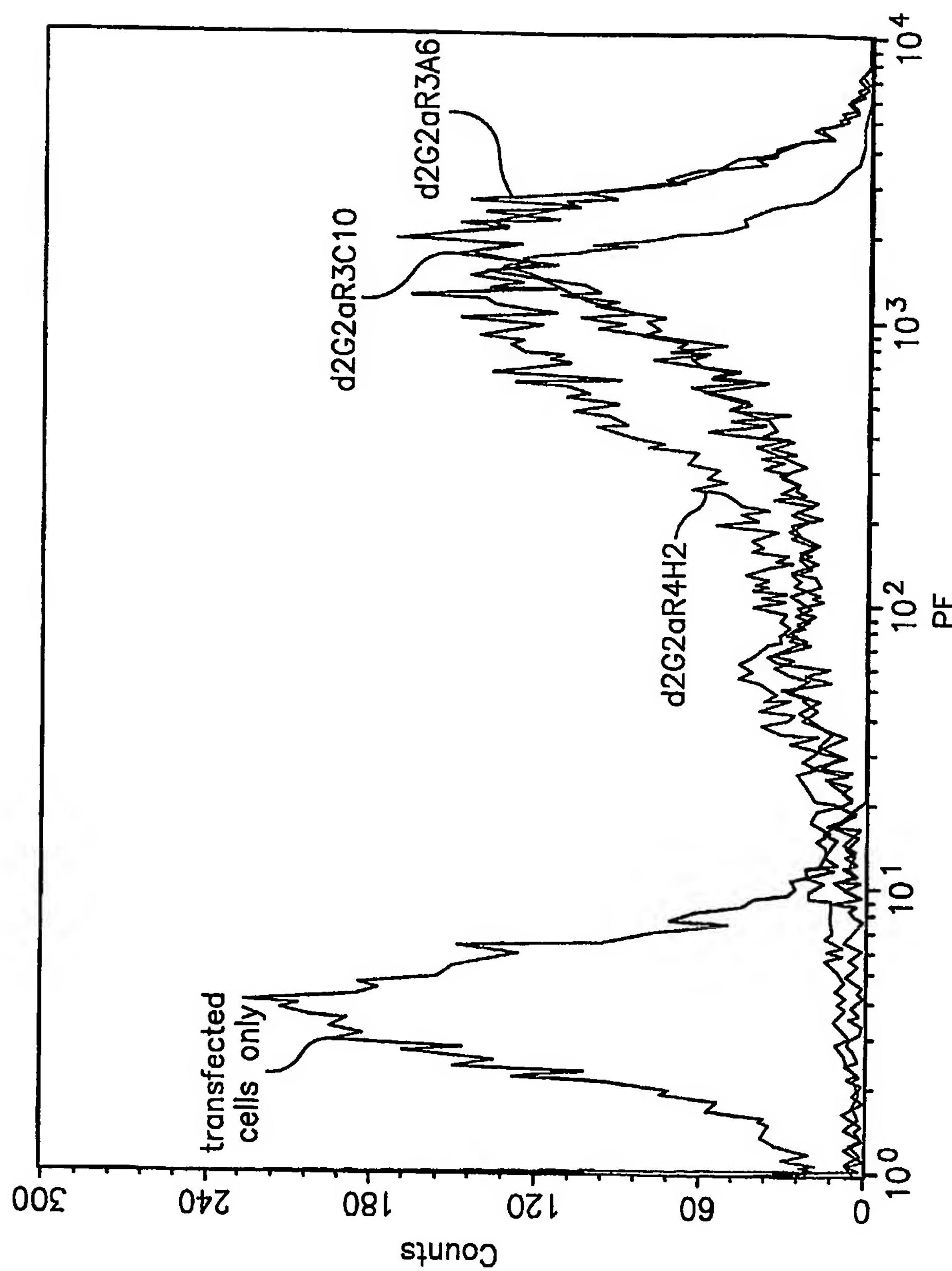


FIG. 18D

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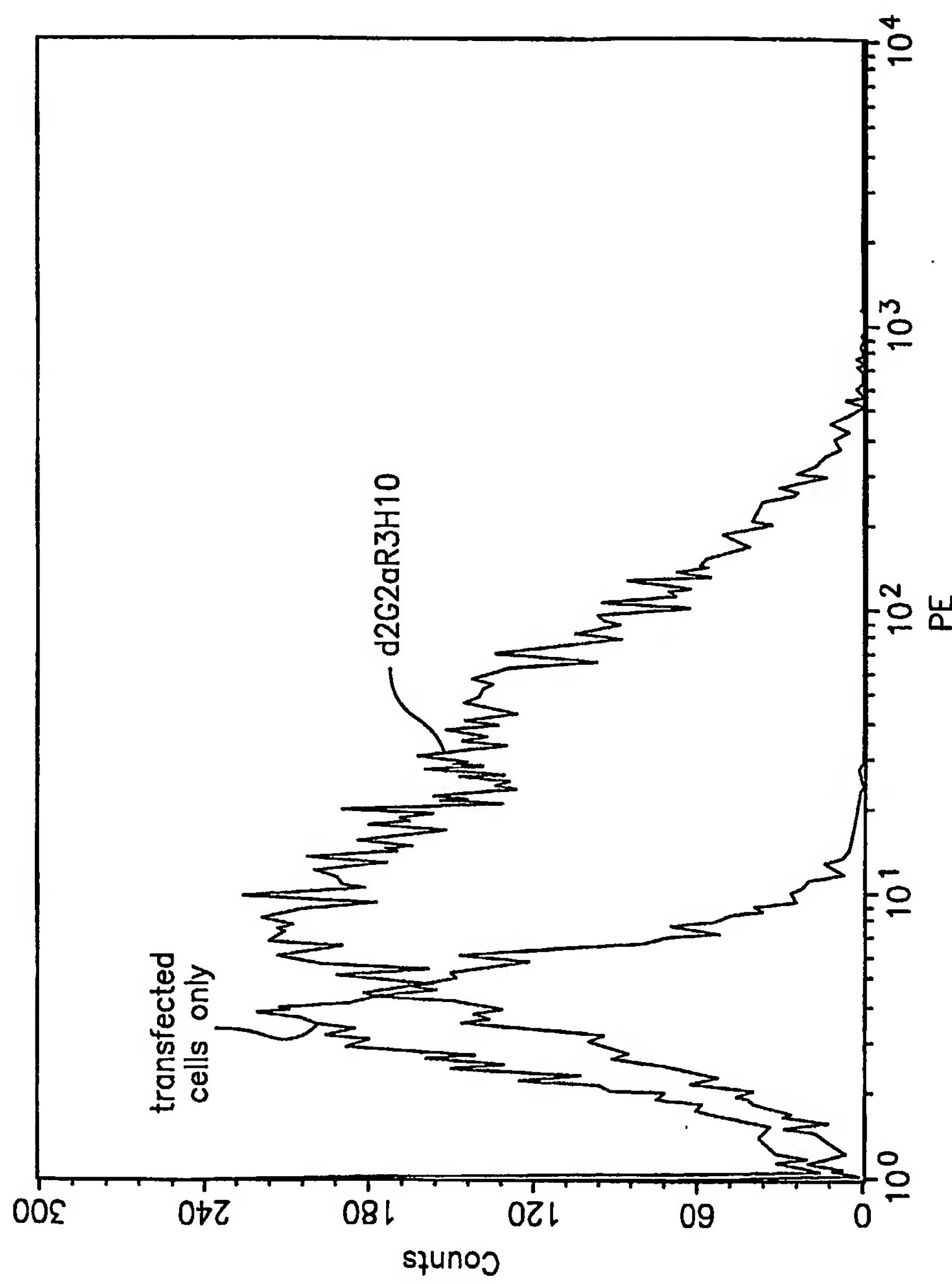


FIG. 18E

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## FLJ32028 binding clones

Fab		FR1	CDR1	FR2	CDR2
c2G1R3A3	LEVQLVESGGGLVQPKGSLKLSCAAS	GFnENTYAMN	WVRQSPGKGLEWVA	RIRTKSNNYATYYADSVKD	
c2G1R3A12	.....	.....	.....	.....	.....
c2G1R3C2	.....	.....	.....	.....	.....
c2G1R3E3	.....	.....	.....	.....	.....
c2G1R3F5	...K	.....	.....	.....	.....
c2G1R3F6	...K	.....	.....	.....	.....
d2G1R4B6	.....	.....	.....	.....	.....
c2G1R4C9	.....	.....	.....	.....	.....
c2G1R3B6	LEVQLQQSGAELAKPGASVKMSCKAS	GYTFITNSWIH	WVKQRPQGQGLEWIG	YIHPGPGYTEYNQNFKD	
c2G1R4G2	LEVOLKQSGAELVKPGASVKKLSCTAS	GFnIKDTYMN	WVKQRPEQGLEWIG	GIDPANDNTYEYPKFQG	
C2G1R4A3	.....	.....	.....	.....	.....
d2G1R4B12	LEVQLQQSGAELVRPGASVTILSCKAS	GYTFTDYEMH	WVKQTPVHGLEWIG	GIDPETGGTVYNQFKKG	
d2G1R4B8	.....	.....	.....	.....	.....
d2G2aR3A6	LEVQLVESGGGLVQPKGSLKLSCAAS	GFTENTYAMN	WVRQAPGKGLEWVA	RIRTKSNNYATYYADSVKD	
d2G2aR3C10	.....	.....	.....	.....	.....
d2G2aR4A12	.....	.....	.....	.....	.....
d2G2aR4G6	.....	.....	.....	.....	.....
d2G2aR4H2	.....	.....	.....	.....	.....
d2G2aR3H10	LEVQLQQSGAELVRPGASVTILSCKAS	GYTFTDYEMH	WVKQTHVHGLEWIG	GIDPETGGTVYNQFKKG	

FIG. 19

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Top sequence in each group is used as a reference for others in the same group. Only the amino acids different from the reference sequence are shown in each sequence and the same amino acids are indicated by dots. ELISA is OD405 reading at 60 min to the FLJ32028 coated wells ( $4 \mu\text{g/ml}$ ) and FACS (geo mean) is showing geometric mean.

**FIG. 19** (Cont.)